L2 ANSWER 1 OF 12 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1

ACCESSION NUMBER: 2000129135 EMBASE

TITLE: An in vitro system for the enzymological analysis of avian hepatitis B virus replication and inhibition in core particles.

AUTHOR: Urban S.; Urban S.; Tyrrell D.L.

CORPORATE SOURCE: S. Urban, MRC Laboratory of Molecular Biology, Trinity College, Cambridge, United Kingdom. sin@mrc-lmb.cam.ac.uk

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030 Pharmacology
004 Microbiology
048 Gastroenterology

LANGUAGE: English

SUMMARY LANGUAGE: English

AB A detailed analysis of the hepatitis B virus (**HBV**) replication reaction is important both in understanding viral biology and in developing effective antiviral drugs. This can best be achieved by studying the viral reverse transcriptase (RT) in its natural context, encapsidated within viral **core** particles in a multiprotein complex, rather than as an isolated enzyme. In order to facilitate a precise enzymological analysis of the avian **HBV**-RT reaction and its inhibition within replicating **cores**, a scheme for the **purification** and analysis of intracellular **core** particles derived from infected liver tissue has been devised, optimized and evaluated. The **purification** scheme itself is simple and rapid, and results in preparations with a 25-fold increase in endogenous polymerase activity that persists for over 5 h under assay conditions. In order to assess the suitability of these preparations for mechanistic studies, a thorough evaluation of purity was undertaken, revealing predominantly pure viral protein and nucleic acid, free of contaminating cellular polymerases and phosphatase activities that potently degrade nucleotides and antiviral drugs. Parameters governing optimal polymerase activity have been determined, and an assay for DHBV-RT activity has been developed which offers the highest purity and specific polymerase activity currently available to study hepadnaviral replication and inhibition.

L2 ANSWER 3 OF 12 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 3
ACCESSION NUMBER: 96309455 EMBASE
DOCUMENT NUMBER: 1996309455
TITLE: In situ DNA polymerase and RNase H activity gel assays as applied to hepadnavirus particles.
AUTHOR: Oberhaus S.M.; Newbold J.E.
CORPORATE SOURCE: Department of Neurology, Univ. of Colorado Hlth. Sci. Ctr.,Denver, CO 80262, United States
SOURCE: Methods in Enzymology, (1996) 275/- (328-347).
ISSN: 0076-6879 CODEN: MENZAU
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We have devised a reproducible system for (1) isolating hepadnavirus particles from the livers and sera of infected animals, and (2) detecting DDDP. RT, and RNase H activities predicted to be encoded by the viral pol gene. In both partially and fully denaturing DNAp activity gels, two major size groups of DNA polymerase (DDDP and RT) activities have been detected with all preparations of DHBV **core** particles and virions at ca. 84-115 kDa (Group 1) and ca. 64-84 kDa (Group 11). In many cases a third size group has been observed ca. 46-60 kDa (Group III), and occasionally DDDP bands have been detected ca. 33-44 kDa (Group IV). These results indicate that the DHBV **core** particle- and virion-associated DNA polymerase (DNAp) activities are consistent among numerous preparations of virus particles. In contrast to the observations of others, these DNAp activities appear to be remarkably resilient since they can be renatured and detected in the gel assays even after boiling and electrophoresis under fully denaturing conditions. The differences in these findings may be related to our use of much larger quantities of **core** particles derived from DHBV-infected livers rather than **HBV**-like particles secreted in vitro from a stably transfected cell line. In addition, we have detected a single, ca. 34- to 36-kDa RNase H activity in association with highly purified DHBV **core** particles and virions isolated from sera taken from DHBV-infected ducks and WHV-infected woodchucks. We have not been able to accurately quantitate the number of virus particles with which the polymerase and RNase H activities we detect are associated, but these activities do not appear to dilute linearly with dilutions of the preparations of virus particles. Additional problems with the **purification** of active Pol proteins may be related to (1) their stability; (2) the possibility that multiple Pol proteins form a complex or secondary structures required for activity, but easily denatured: or (3) the possibility that the Pol protein(s) requires other viral or cellular factors for activity. Despite many attempts, we have been unable to characterize the DHBV-associated DNAp and RNase H activities using a panel of anti-DHBV Pol peptide antisera and Western blot analysis, or immunoprecipitation and activity gel assays. However, the reproducible detection of DNAp and RNase H activities in (1) preparations of intracellularly derived **core** particles purified to near homogeneity from the livers of infected ducks and (2)

extracellular virions derived from the sera of infected ducks and woodchucks supports the idea that these activities reside within virus particles and are likely to be products of the viral pol gene. It is possible that the less-than-full-length DNAp and RNase H activities result from proteolytic degradation of the protein(s) retained within, or released from, **core** particles and virions or that they are host derived and present in preparations of virus particles as a result of adhering to virus particles. We believe that both of these possibilities are unlikely. The reproducible detection of the same DNAp and RNase H activities in numerous preparations of virus particles derived from sources as diverse as infected avian liver tissue and sera from infected woodchucks does not support their generation or presence by mechanisms as inconsistent as random proteolysis or the adventitious adherence of intracellular host enzymes to particles which are rigorously purified to near homogeneity. Although our data suggest the possibility that multiple pol gene products are present, there have been no reports of studies in which less-than-full- length Pol proteins containing minimal predicted RT or RNase H domains have been heterologously expressed and tested for enzymatic activity. The long- awaited availability of an in vitro system in which a hepadnaviral protein(s) with enzymatic activity is expressed has confirmed the role of a pol gene product in the priming and reverse transcription of minus-strand DNA and has the potential to elucidate other details about the hepadnavirus polymerase. The inability of these full-length pol gene products to complete minus- strand synthesis suggests that the nature of the hepadnaviral replicase and the conditions required for its full repertoire of proposed activities have not yet been clearly defined. Although activity gel analyses of hepadnavirus- associated polymerase and RNase H activities are not convenient, their reproducibility and the lack of any other assay system in which to study these activities underscore the potential of these assays for their characterization. In addition, the results of these assays suggest possible alternative approaches to improve the expression of active pol gene products in vitro.

L2 ANSWER 4 OF 12 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 4
ACCESSION NUMBER: 95299474 EMBASE
DOCUMENT NUMBER: 1995299474
TITLE: The hepatitis nucleocapsid as a vaccine carrier moiety.
AUTHOR: Milich D.R.; Peterson D.L.; Zheng J.; Hughes J.L.; Wirtz R.; Schodel F.
CORPORATE SOURCE: Department of Molecular Biology, The Scripps Research Institute, 10666 North Torrey Pines Road,La Jolla, CA 92037, United States
SOURCE: Annals of the New York Academy of Sciences, (1995) 754/- (187-201).
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COUNTRY: United States
DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The 'carrier effect', defined as the provision of T cell recognition sites physically linked to B cell epitopes in order to provide Th cell function for antibody synthesis, is well known. Peptides, proteins, and more recently particulate protein antigens have been used for this purpose. The hepatitis B **core** antigen represents a highly immunogenic antigen antigen in humans as well as in experimental animal models. Studies in mice have provided insight into this enhanced immunogenicity. For example, HBcAg directly activates B cells (i.e., T cell independence), HBcAg elicits strong T cell responses, and HBcAg is efficiently processed and presented by antigen presenting cells (APCs). These characteristics suggested that HBcAg may be an ideal carrier moiety for B cell epitopes requiring additional Th cell function. Therefore, a number of **HBV** and non-**HBV** B cell epitopes have been chemically linked or fused by recombinant methods to HBcAg as a method to increase immunogenicity with significant success. We have designed bacterial expression vectors that allow insertion of heterologous B cell epitopes at various positions within HBaAg particles and permit efficient **purification** of hybrid HBcAg particles. Studies of positional effects have demonstrated that an internal insertion into a dominant HBcAg-specific B cell site represents a superior location for enhanced antibody production. Immunogenicity studies have been extended to protection against experimental challenge in several systems. For example, a malaria CS repeat sequence derived from P. berghei was inserted into HBcASg at the internal site, and purified hybrid HBcAg/CS particles were highly immunogenic and protected 100% of experimentally challenged BALB/c mice. This system has also been exploited for purposes of oral vaccination by expressing genes coding for hybrid HBcAg particles in live, avirulent vaccine strains of Salmonella species.